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A native multi-dimensional monitoring workflow for at-line characterization of mAb titer, size, charge, and glycoform heterogeneities in cell culture supernatant

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Introduction

A native-multi-dimensional-muti attribute monitoring (n-MD-MAM) workflow that can be applied as a Process Analytical Technology (PAT) enabler and offer information on harvest titer, size variants, charge variants (of individual size variants) as well as intact glycoform profile for each charge variant within a single workflow. This has been achieved through extension of the 1st dimension through coupling of ProA in series with size exclusion chromatography (SEC), followed by weak cation exchange (WCX) chromatography in the 2nd dimension. The method facilitates in-process analysis of biologics and can be applied directly to cell culture supernatant without the otherwise pre-requisite polishing step. The proposed workflow has been shown to be compatible with routine sample matrices, thus enabling characterization of drug substance (DS) and drug product (DP).

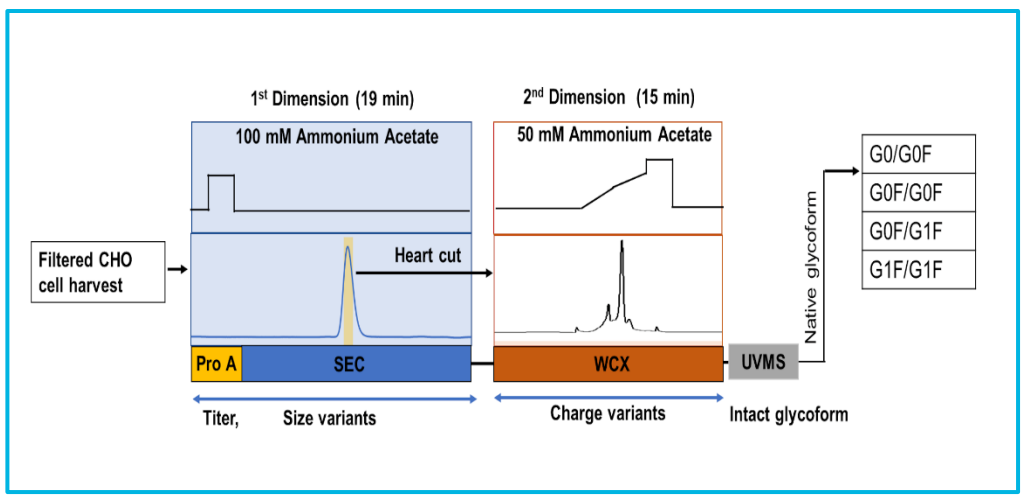

Figure 1. Native multi-dimensional MAM workflow for at-line characterization of mAb titer, size, charge, and glycoform heterogeneities.

Experimental

Materials & methods

Cell culture supernatant (CHO supernatant in CDCHO media) of trastuzumab expressed in-house and purified DP has been used for of the proposed n-MD-MAM workflow.

1D ProA in series with size exclusion chromatography (SEC)

Bio-Monolith rProteinA column (5.2 x 5mm, Agilent Technologies, Santa Clara, California, United States) was coupled in series with BioCore SEC-300 column (5µm, 7.8x300 mm, NanoChrom). Binding was achieved at 100% A between 0 to 0.6 min followed by elution with 100% B till 1.8 min. The re-equilibration step (100%A) was extended to 19 min to facilitate resolution in the SEC phase of the 1st dimension (Table 1).

2D-weak cation exchange chromatography (WCX)

For separation of heart cut peaks in the 2D, BiomAb NP5, PK 5µm nonporous (4.6 x 250 mm, Agilent Technologies, Santa Clara, California, United States) was employed. Buffer composition consisted of 50 mM ammonium acetate at 6.8 (Buffer A) and 10.11 (Buffer B) at 30°C. Separation was achieved through a step gradient with a linear increase in % B up to 56% till 8.60 min followed by an increase of %B from 56% (8.60 min) to 70% (11.00 min) and was monitored at 280 and 220 nm, respectively.

Table 1. Comparable column performance between (a) nonvolatile and (b) volatile buffer systems for rProA separation. (c) ProA-SEC workflow where equilibration step of rProA continued as an isocratic separation step for SEC column and monomer peak eluted through.

Type of separation	Flow rate (mL/min)	Injection volume (µg)	Buffer A	Buffer B	Run time (min)	Retention time (min)	Area (mAU*min)
ProA	1.00	25.00	15 mM Sodium phosphate (pH 7.40)	100mM Citric Acid (pH 2.60)	4.00	1.40 (±0.01)	365.52 (±3.46)
ProA	1.00	25.00	100 mM Ammonium Acetate (pH 6.80)	100mM Ammonium Acetate (pH 3.00)	4.00	1.68 (±0.03)	365.50 (±2.01)
ProA-SEC	1.00	25.00	100 mM Ammonium Acetate(pH 6.80)	100mM Ammonium Acetate(pH 3.00)	19.00	9.72 (±0.01)	365.59 (±4.66)

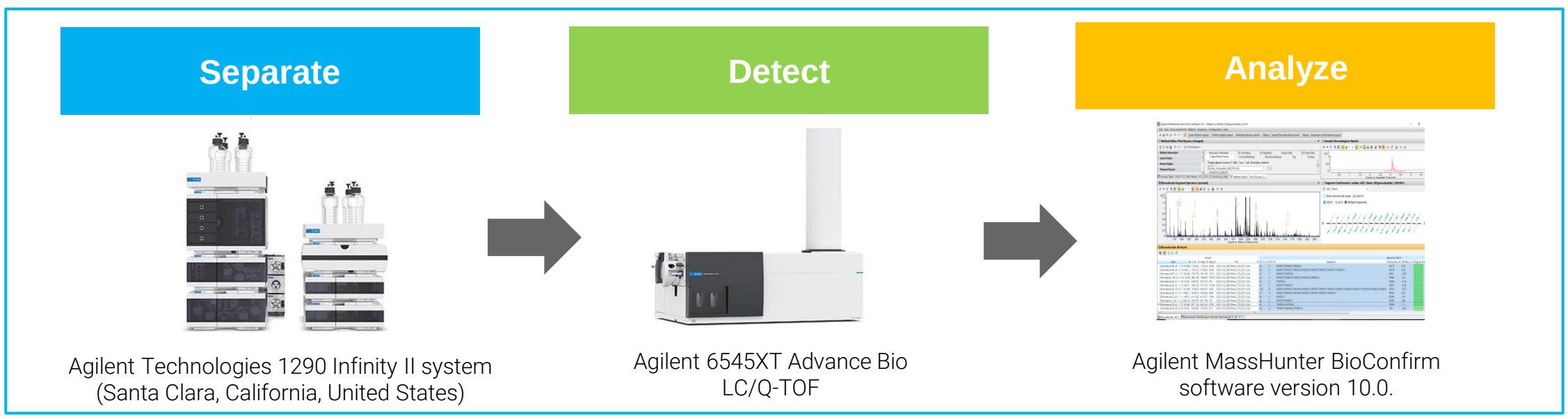

Figure 2. Instrumentation used for at-line characterization of mAb titer, size, charge, and glycoform heterogeneities in cell culture supernatant.

In-series coupling of Pro-A and SEC (1st dimension)

1D workflow for in series ProA-SEC was designed, consisting of 3 phases 1) ProA binding phase (pH 6.8), 2) ProA elution phase (pH 3), and 3) SEC separation phase (pH 6.8). As eventual method compatibility with MS was desired, the 1D workflow was developed in a volatile MS compatible buffer system consisting of 100 mM ammonium acetate (1). Additionally, as the detector in the 1st dimension appears post SEC and quantification is based on sample UV absorbance on SEC, for titer determination, efficient sample transfer from ProA to SEC was also established (Table 1).

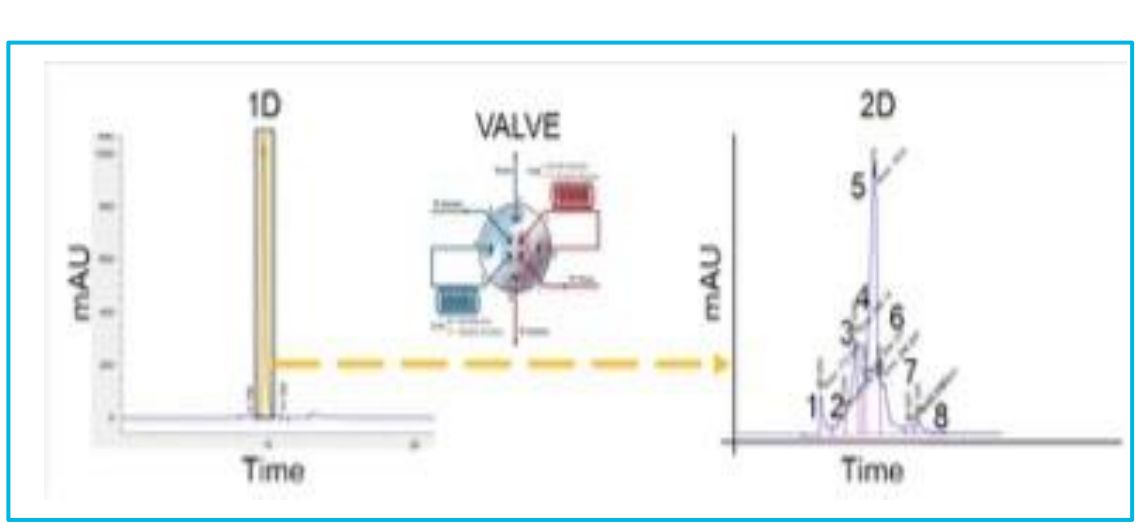

Figure 3. 1D represent 1st dimension where rProA is tagged with SEC and 2D represents WCX separation. 1D and 2D are connected via a 2D-LC comprehensive valve to transfer the heart-cut fraction to the second dimension.

Charge variant separation using weak cation exchange (2nd dimension)

The overall method for 2nd dimension hyphenated with MS was 30 minutes at a flowrate of 0.4 ml/min (2). The heart cut from 1st dimension resolved into 8 charge variants in the 2nd dimension (Figure 3).

Aoolication: Characterization of trastuzumab Pro-A in series SEC (1D) - WCX (2D) - MS

As a PAT application of the n-MD-multi-attribute monitoring workflow described along with MS, trastuzumab, was characterized in cell culture supernatant and compared with in-formulation DP (Figure 4) (Table 2 & Table 3).

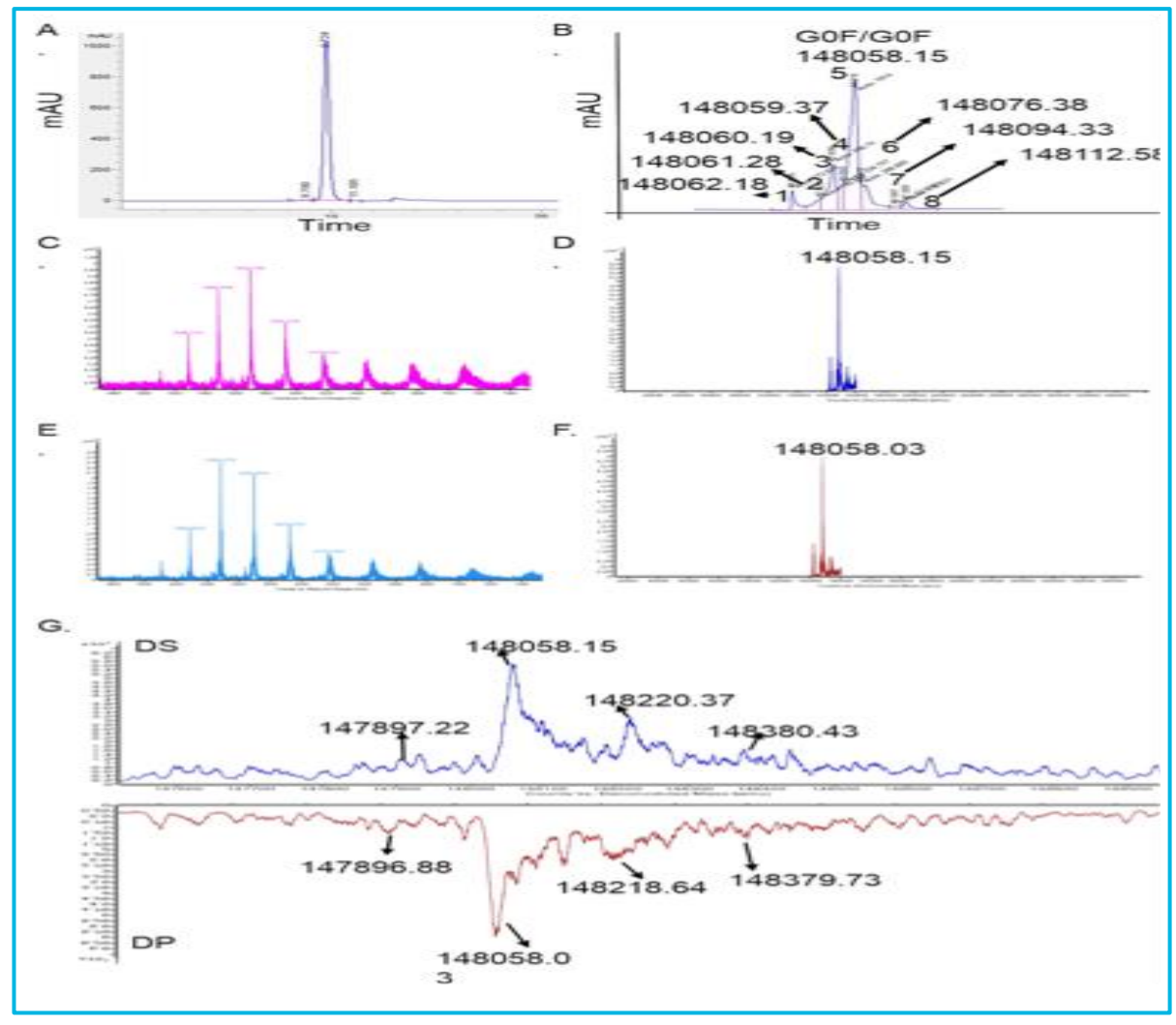

Figure 4. rProA-SEC-WCX-MS analysis of Trastuzumab: (A) SEC chromatogram using the UV detector; (B) WCX chromatogram with UV detection; Spectrum plot of DS (C) and DP (E) to confirm ionization under native form; Deconvolution of charge variants peaks of DS (D) and DP (F); (G) Deconvoluted glycoform mirror plot of DS and DP.

Table 2. n-MD-MAM workflow for PAT application: characterization and comparison of cell culture supernatant (DS) and in-formulation product (DP) of trastuzumab

DS						
1D: ProA-SEC					2D: WCX	
Peak	Retention time	Area %	1D cut start time	2D run start time	peak no	Area%
1 (pre peak 1)	8.79 (±0.01)	0.46 (±0.02)				
2 (main peak)	9.72 (±0.04)	99.42 (±0.01)	9.7 (±0.00)	57.56 (±0.00)	1 (pre peak 4)	5.30 (±0.10)
3 (post peak)	11.10 (±0.02)	0.12 (±0.00)			2 (pre peak 3)	4.47 (±0.00)
					3 (pre peak 2)	17.42 (±0.11)
					4 (pre peak 1)	6.29 (±0.10)
					5 (main peak)	49.62 (±0.03)
					6 (post peak 1)	11.94 (±0.52)
					7 (post peak 2)	1.65 (±0.51)
					8 (post peak 3)	3.28 (±0.52)
DP						
1D: ProA-SEC					2D: WCX	
Peak	Retention time	Area %	1D cut start time	2D run start time	peak no	Area%
1 (pre peak 1)	8.78 (±0.00)	0.35 (±0.00)				
2 (main peak)	9.71 (±0.07)	99.56(±0.04)	9.7 (±0.00)	56.45 (±0.01)	1 (pre peak 4)	2.02 (±0.23)
3 (post peak)	11.66 (±0.12)	0.09 (±0.05)			2 (pre peak 3)	4.28 (±0.21)
					3 (pre peak 2)	20.23 (±0.24)
					4 (pre peak 1)	4.10 (±0.23)
					5 (main peak)	58.49 (±0.10)
					6 (post peak 1)	7.91 (±0.05)
					7 (post peak 2)	1.44 (±0.05)
					8 (post peak 3)	1.53 (±0.04)

Results and Discussion

Table 3.. The Analysis of mass of monomer, pre-peak 1 (high molecular weight species: HMWS) and post-peak 1(low molecular weight species: LMWS) of 2nd dimension resolved in the corresponding glycoforms and modifications.

MONOMER ANALYSIS (spectra 2000-7000)												
		Acidic variants				Main peak				Basic variants		
SAMPLE NAME	CHARGE ENVELOPE	A4	A3	A2	A1					B1	B2	B3
Harvest (DS)	46-73	148062.18 (± 1.18)	148061.28 (±1.65)	148060.19 (±1.59)	148059.37 (±1.64)	147897.22 (±1.58)	148058.15 (±1.48)	148220.37 (±0.49)	148380.43 (±1.59)	148076.38 (±0.96)	148094.33 (±1.73)	148112.585 (±1.49)
		4 DEAMIDATION	3 DEAMIDATION	2 DEAMIDATION	1 DEAMIDATION	G0/G0F	G0F/G0F	G0F/G1F	G1F/G1F	OXIDATION	OXIDATION	3 OXIDATION
Control (DP)	42-71	148062.70 (± 0.64)	148061.84 (±1.15)	148060.02 (±0.49)	148059.08 (±1.03)	147896.88 (±0.53)	148058.03 (±0.98)	148218.64 (±0.70)	148379.73 (±0.00)	148075.75 (±1.00)	148092.86 (±2.13)	148111.64 (± 0.89)
		4 DEAMIDATION	3 DEAMIDATION	2 DEAMIDATION	1 DEAMIDATION	G0/G0F	G0F/G0F	G0F/G1F	G1F/G1F	OXIDATION	OXIDATION	3 OXIDATION
HMW SPECIES (PRE PEAK 1) ANALYSIS (spectra 5000-12000)												
			Acidic variants			Main peak				Basic variants		
SAMPLE NAME	CHARGE ENVELOPE		A3	A2	A1					B1	B2	B3
Harvest (DS)	35-59		296122.02 (±1.08)	296118.43 (±1.45)	296116.56 (±1.83)	295792.93 (±1.07)	296114.58 (±1.58)	296438.77 (±1.23)		296178.23 (±1.77)	296210.64 (±0.99)	296242.01 (±0.45)
			4 DEAMIDATION	2 DEAMIDATION	1 DEAMIDATION	G0/G0F	G0F/G0F	G1F/G0F		OXIDATION	OXIDATION	4 OXIDATION
Control (DP)	31-59		296122.98 (±1.27)	296118.77 (±1.22)	296116.06 (±1.83)	295792.88 (±0.87)	296114.25 (±0.49)	296438.62 (±1.29)	296758.47 (± 1.41)	296180.58 (±1.14)	296210.14 (±1.76)	296248.24(± 1.37)
			4 DEAMIDATION	2 DEAMIDATION	1 DEAMIDATION	G0/G0F	G0F/G0F	G1F/G0F	G1F/G1F	OXIDATION	OXIDATION	4 OXIDATION
LMW SPECIES (POST PEAK 1) ANALYSIS (spectra 500-4000)												
						Main peak				Basic variants		
										B1	B2	
SAMPLE NAME	CHARGE ENVELOPE											
Harvest (DS)	19-36					73948.22 (±0.33)	74029.07 (±0.69)	74109.38(±0 .37)		74038.75(± 1.25)	74045.38(±1.22)	
						G0	G0F	G1F		OXIDATION	OXIDATION	
Control (DP)	25-36					73948.55(±1.53)	74028.38(±0.36)	74108.64 (±0.47)		74036.94 (± 0.09)	74044.1 0(±0.79)	
						G0	G0F	G1F		OXIDATION	OXIDATION	

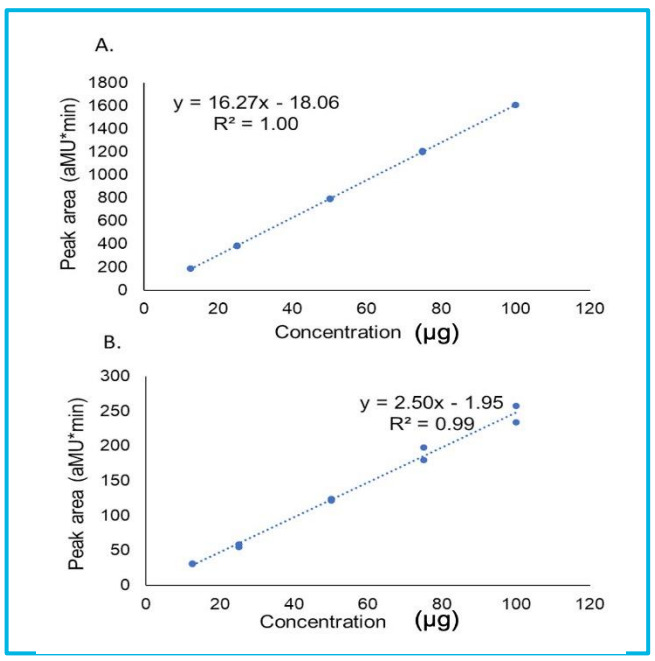

Figure 5. Linearity and standard curve determination to enable titer estimation through Pro-A in series SEC and WCX

Method validation

To facilitate titer estimation, efficient transfer of eluted mAb from ProA to SEC column in series was determined followed by determination of LOD and LOQ. Linearity, LOD, LOQ, and standard curve were established to enable titer estimation through Pro-A in series SEC and WCX (Figure 5). An LOD of 0.50 µg and LOQ of 1.521 µg was obtained for the main peak 1st dimension and 6.512 µg and 19.734 µg for the main peak in 2nd dimension.

Emphasize

- Single heart cut could be completed in 25 min (Fig 1).
- Method can be applied as a Process Analytical Technology (PAT) enabler, without pre-requisite polishing step.

Conclusions

The new generation of analytical workflows are faster, more robust, and increasingly efficient with the ability to monitor multiple attributes within a single workflow, in the direction of creates unique PAT opportunities for at-line/off-line in-process analysis towards expedition of product development and facilitation of more informed decision making in shorter time.

References

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